

# Heat shock protein 90 as a molecular target for cancer therapeutics

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## Introduction

Cancer is a disease characterized by genetic instability. Although identification of novel therapeutic agents via molecular targeting offers the promise of great specificity coupled with reduced systemic toxicity, specific inhibition of individual proteins or signaling pathways faces the potential peril of being subverted by the inherent genetic plasticity of cancer cells. Cancer cells are very adept at adapting to noxious environments. Thus, hormone-dependent tumors eventually become hormone-independent, either by receptor mutation or via activation of alternative pathways leading to receptor stimulation. Similarly, cancer cells exposed to initially fatal levels of chemotherapy eventually activate multiple and overlapping signaling pathways to protect themselves from further harm, while tumors deprived of oxygen upregulate a multifaceted transcriptional response that allows them to cope successfully with the hypoxic state.

If one assumes that cancer cells are always under moderate to severe stress of one type or another, an approach to this apparent dilemma might be to target the basic machinery that allows cancer cells to adapt so successfully to stress. Cells respond to stress by increasing synthesis of a number of molecular chaperones (also known as heat shock proteins, or Hsps, because they were first observed in cells exposed to elevated temperature). These housekeeping proteins, as their name implies, assist general protein folding and prevent nonfunctional side reactions such as the nonspecific aggregation of misfolded or unfolded proteins. However, within the last decade, one chaperone in particular, heat shock protein 90 (Hsp90), has emerged as being of prime importance to the survival of cancer cells. Hsp90 is constitutively expressed at 2- to 10-fold higher levels in tumor cells compared to their normal counterparts, suggesting that it may be critically important for tumor cell growth and/or survival. A small molecule inhibitor of Hsp90, the benzoquinone ansamycin 17-allylamino-17-desmethoxygeldanamycin (17-AAG), has shown antitumor activity in several human xenograft models, including colon, breast, and prostate cancer (Basso et al., 2002; Kelland et al., 1999; Solit et al., 2002). The drug is currently completing multi-institution phase I clinical trials, and phase II trials are being planned. Other Hsp90 inhibitors are also at various stages of development. For a detailed review of Hsp90 inhibitor drug development, including a discussion of pharmacodynamic endpoints, see the recent review by Maloney and Workman (2002). Why has this molecular target garnered so much recent interest?

## What happens upon Hsp90 inhibition?

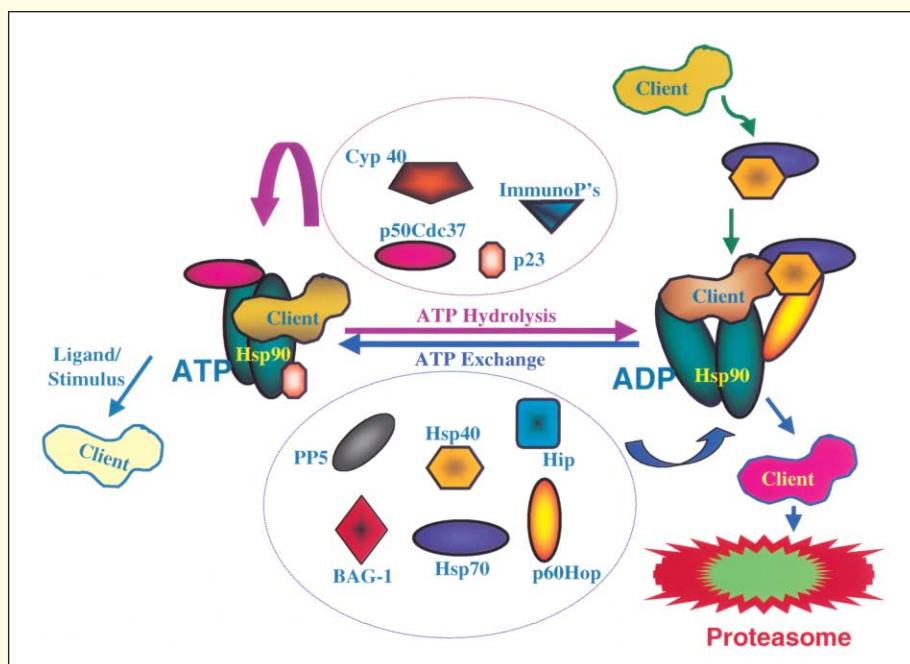
Discussion of Hsp90 function cannot be separated from a discussion of its small molecule inhibitors, particularly the benzoquinone ansamycin geldanamycin (GA), the first inhibitor of Hsp90 to be identified. When Whitesell et al. first identified Hsp90 as the intracellular protein to which GA binds, it was

unclear what effect drug binding had on chaperone activity (indeed, the full extent of Hsp90 function was not appreciated) (Whitesell et al., 1994). For several years prior, GA and related benzoquinone ansamycins such as herbimycin A had been used as tyrosine kinase inhibitors. In the initial report of GA binding to Hsp90, we made the observation that, while GA did not directly affect the kinase activity of p60<sup>v-src</sup>, drug addition to v-src-transformed 3T3 cells dramatically shortened the half-life of the protein without affecting its mRNA level or translation rate, thus effectively reducing kinase activity by reducing the intracellular content of kinase protein. An association of p60<sup>v-src</sup> with Hsp90 had been described in the early 1980s, although the reason for this association was not known (Oppermann et al., 1981). In our report describing GA binding to Hsp90 and the concomitant destabilization of v-src protein, we also observed a rapid, apparent dissociation of p60<sup>v-src</sup> from Hsp90 in drug-treated cells, and we reasoned that this led to p60<sup>v-src</sup> instability (Whitesell et al., 1994). Later studies revealed that the association of client proteins, such as p60<sup>v-src</sup>, with Hsp90 was in fact not fully lost in the presence of GA, but altered to become more sensitive to detergent, and that GA-induced protein destabilization was due to ubiquitination-dependent, proteasome-mediated protein degradation (Schneider et al., 1996). Thus, the paradigm of the effects of Hsp90 inhibition on its client proteins (e.g., p60<sup>v-src</sup>)—drug-induced alteration in Hsp90 association followed rapidly by polyubiquitination and proteasome-mediated degradation—was already apparent in these initial studies.

The list of Hsp90 client proteins has grown strikingly over the last several years, and it includes key components of multiple signaling pathways utilized by cancer cells for growth and/or survival (for a review, see Neckers, 2002). Hsp90 clients are not limited to kinases, although a large number of kinases (e.g., HER2, AKT, c-RAF-1) are clients. Ligand-dependent transcription factors (e.g., steroid receptors), transcription factors (e.g., HIF-1 $\alpha$ ) containing PAS domains ("PAS" delineates a common motif shared by the transcription factors PER, ARNT, and SIM), and, importantly, mutated or chimeric signaling proteins (mutated p53, NPM-ALK kinase, p210<sup>Bcr-Abl</sup>) are also Hsp90 clients. While certain motifs to which Hsp90 binds (e.g., PAS domains) have been described, there is no single Hsp90 binding motif, and the rules that determine a requirement for Hsp90 association remain elusive. Because GA binding to Hsp90 uniformly results in client protein destabilization, GA has proven to be an invaluable tool in expanding the list of Hsp90-dependent signaling proteins. As the number of client proteins grows, it seems that a characteristic shared by many is a functional necessity to form multiprotein complexes. Whether this proves to be a unifying motif in identifying Hsp90 client proteins remains to be determined.

## How does Hsp90 function?

Why does GA almost uniformly cause the destabilization of Hsp90 client proteins? This requires an understanding of Hsp90



**Figure 1.** Nucleotide-dependent cycling of the Hsp90-based super-chaperone machine

Hsp90 forms the basis of a super-chaperone machine that promotes the proper folding of client proteins so that they can respond to a stimulus or bind ligand. However, the machine is in constant flux and cycles between two Hsp90 conformations, determined by nucleotide binding, which in turn specify which set of cochaperones associate with the chaperone complex. Cochaperones that can associate with one conformation or the other include p23, p50Cdc37, p60<sup>Hop</sup>, immunophilins, cyclophilins, Hsp70, Hip, phosphatase PP5, Hsp40, and BAG-1. Cycling of this machine is driven by ATP hydrolysis. Although Hsp90 is a weak ATPase, its activity is regulated by cochaperones and dramatically enhanced by client protein binding. A client protein's half-life may be stochastically determined by the length of time it resides in association with the Hsp90-Hsp70 form of the chaperone machine, because at this time, the client protein is susceptible to ubiquitination and delivery to the proteasome.

is in the receptive conformation, the chaperone cycle will reverse. Upon ATP hydrolysis, Hsp90 conformation alters, releasing those cochaperones that asso-

function. GA and other Hsp90 inhibitors (e.g., radicicol; Sharma et al., 1998) have not only been used extensively to identify Hsp90 client proteins, but also as probes of Hsp90 function. To this end, several laboratories simultaneously determined that GA bound to Hsp90 in a structurally unique amino terminal pocket (Grenert et al., 1997; Prodromou et al., 1997; Stebbins et al., 1997). Biochemical and structural studies (Grenert et al., 1997; Prodromou et al., 1997) identified this pocket as a novel nucleotide binding domain, thus resolving a long-standing controversy as to whether Hsp90 in fact bound ATP and possessed ATP hydrolyzing activity. As early as 1993, Csermely had suggested that ATP bound to Hsp90 and altered its conformation (Csermely et al., 1993). Although this idea was initially met with some skepticism, the studies described above, as well as others from the laboratories of David Toft and Lawrence Pearl, finally confirmed the importance of ATP/ADP binding and ATP hydrolysis to the function of Hsp90 (Grenert et al., 1999; Panaretou et al., 1998).

A model of Hsp90 function has thus emerged in which nucleotide binding to the amino terminal pocket alters Hsp90 conformation sufficiently to define distinct, nonoverlapping subsets of cochaperone proteins with which Hsp90 interacts, thus forming a "super-chaperone machine" that cycles between at least two conformations (Scheibel and Buchner, 1998). An Hsp90 client protein first associates with an Hsp70/Hsp40 chaperone complex (Hernandez et al., 2002). This assemblage is next linked to Hsp90 via p60<sup>Hop</sup>, an Hsp90/Hsp70 interacting protein. At this point, when the client protein is being loaded on Hsp90, the chaperone is in its ADP-bound conformation. Replacement of ADP by ATP in Hsp90 alters its conformation, releasing p60<sup>Hop</sup> and the Hsp70/Hsp40 complex, and recruiting another set of cochaperones, including p23 and certain immunophilins or p50<sup>Cdc37</sup>. This association of cochaperones with Hsp90 folds and stabilizes client proteins, and temporarily holds them in a state that can bind ligand (e.g., steroid hormones) or respond to a stimulus (e.g., cytosolic kinases). However, this machine is neither static nor unidirectional. If a client protein fails to receive its stimulus or see its ligand while it

associate with its ATP-bound state and recruiting those cochaperones that prefer to associate with Hsp90's ADP-bound conformation. Although the ATPase activity of Hsp90 is very weak, it is regulated by both cochaperone association and client protein binding (McLaughlin et al., 2002; Siligardi et al., 2002; Young and Hartl, 2000). Most recently, Panaretou and colleagues have identified a novel Hsp90 cochaperone, which they have termed Aha1. Aha1 binding stimulates the inherent ATPase activity of Hsp90 (Panaretou et al., 2002).

Thus, ATP hydrolysis and ADP/ATP nucleotide exchange drive the cycling of the Hsp90-based super-chaperone machine. While a client protein is associated with the ADP-bound Hsp90-based chaperone complex, it is no longer folded properly to respond to a stimulus or bind ligand. The mechanism by which a client protein is targeted for degradation is not well understood, but may partly depend on the frequency with which it finds itself in the Hsp70 portion of the cycle, for it is at this point that the chaperone machine appears capable of recruiting specific ubiquitinating and proteasome-interacting proteins which serve to redirect the client protein to the proteasome, where it is degraded (see Figure 1).

GA (and radicicol) replaces nucleotide in the Hsp90 binding pocket with an affinity much greater than either ATP or ADP, thus effectively short-circuiting the chaperone cycle much as a stick thrust between the spokes of a bicycle wheel precludes further movement. However, since the GA-bound conformation of Hsp90 resembles the chaperone's ADP-bound conformation, GA binding promotes stable assembly of the super-chaperone machine that favors client protein degradation (Schneider et al., 1996). Thus, in the presence of GA, the half-lives of Hsp90 client proteins are uniformly shortened, so that in some cases their steady-state level becomes undetectable (see Figure 2). Very recently, several groups have identified E3 ubiquitin ligases containing chaperone-interacting motifs. At least one of these proteins, CHIP, has been shown to promote the ubiquitination and degradation of some Hsp90 clients (Connell et al., 2001), including HER-2 (Xu et al., 2002).

## Translation of Hsp90 inhibition to clinical use in cancer

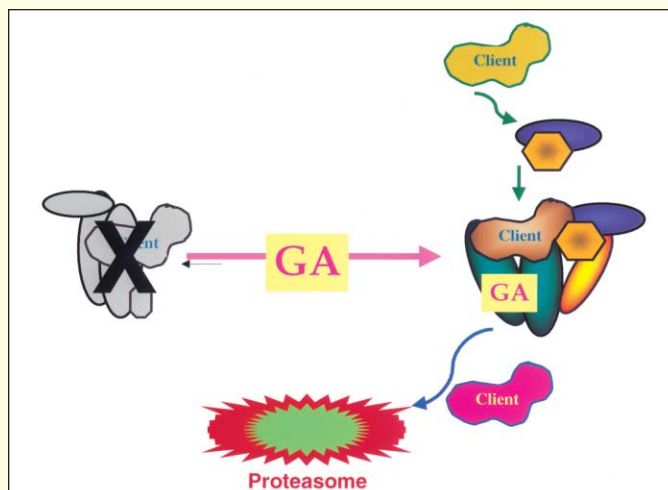
Because of the wide array of Hsp90 client proteins, the translational development of Hsp90 inhibitors is taking many directions. As mentioned above, single agent phase I trials have been run at multiple institutions examining several different schedules of drug administration. Using information gained from these multiple phase I studies, targeted phase I/II combination trials and phase II single agent trials are being developed. The remainder of this review focuses on 3 Hsp90 clients, p210<sup>Bcr-Abl</sup>, HER-2, and HIF-1 $\alpha$ /HIF-2 $\alpha$ , and describes how inhibition of these proteins by targeting Hsp90 with 17-AAG (the derivative of GA used clinically) has been incorporated into 3 distinct clinical studies. Additional phase I/II and phase II studies of 17-AAG alone or in combination with other chemotherapeutics are being planned, and some of these are listed in Table 1.

### 1. Chronic myelogenous leukemia (Hsp90 client: p210<sup>Bcr-Abl</sup>)

Distinct fusion products of the *Bcr* and *Abl* genes, p210<sup>Bcr-Abl</sup> or p185<sup>Bcr-Abl</sup>, have been directly implicated in the development of chronic myelogenous leukemia (CML) and acute lymphocytic leukemia (ALL), respectively. We recently reported that the p210<sup>Bcr-Abl</sup> protein is dependent on association with Hsp90 for its stability and that treatment of cells with GA or 17-AAG leads to rapid destruction of p210<sup>Bcr-Abl</sup> (Blagosklonny et al., 2001). Bhalla and colleagues have since reported that high (5  $\mu$ M) concentrations of either GA or 17-AAG induced apoptosis in CML-derived cell lines, as well as in cells transfected with the *Bcr-Abl* gene (Nimmanapalli et al., 2001). Shiotsu et al. had previously shown that the Hsp90 inhibitor radicicol had a similar proapoptotic effect on p210<sup>Bcr-Abl</sup>-expressing CML cells in vitro, and also significantly prolonged the survival of SCID mice inoculated with K562 cells (Shiotsu et al., 2000). Similar results have been obtained with imatinib (Gleevec), a moderately specific *Bcr-Abl* kinase inhibitor, confirming that inhibition (or degradation) of p210<sup>Bcr-Abl</sup> is sufficient to induce apoptosis in CML. However, it is important to note that CML cells that have become resistant to imatinib in vivo retain their Hsp90 dependence and thus remain sensitive to 17-AAG (Gorre et al., 2002). Based on these data, a study will soon be commencing at Karmanos Cancer Institute (Wayne State University) testing the combination of 17-AAG and imatinib both in patients with accelerated or blastic phase CML (where response to imatinib alone is short-lived), and in chronic phase patients who have failed to achieve a cytogenetic response with imatinib alone.

### 2. HER-2-positive breast and prostate cancer (Hsp90 client: HER-2)

HER-2 is one of the most sensitive of the Hsp90 client proteins to GA and 17-AAG, and both maturing and fully mature forms of the receptor depend on Hsp90 association for stability (Xu et al., 2001). HER-2 overexpression is a common phenomenon in multiple malignancies, and has been associated with development of resistance to both chemotherapeutic and biologic agents (Baselga et al., 1998). Conversely, downregulation of ErbB2 receptor on cells by using ErbB2-specific monoclonal antibodies sensitizes ErbB2-overexpressing cancer cells to chemotherapeutic agents, such as taxol and doxorubicin (Baselga et al., 1998). Furthermore, results of a recent clinical trial demonstrated that the taxol response rate of patients with HER-2-overexpressing breast cancers was significantly higher among those receiving taxol plus herceptin compared to those receiving taxol alone (Pegram et al., 1998). Based on very promising preclinical data in breast and prostate cancer models in which 17-AAG markedly enhanced the toxicity of taxol in the



**Figure 2.** GA short-circuits the cycling of the Hsp90-based super-chaperone machine

When GA or 17-AAG bind in Hsp90's nucleotide pocket, the drugs' high affinity for Hsp90 blocks the cycling of the chaperone machine and alters Hsp90's conformation to promote stable assembly of those components that favor client protein ubiquitination and proteasome delivery.

context of HER-2 overexpression (Basso et al., 2002; Solit et al., 2002), a phase I/II clinical trial is being planned at Memorial Sloan Kettering Cancer Center to test the efficacy of 17-AAG/taxol combination in HER-2 overexpressing breast and prostate cancer.

### 3. VHL-negative clear cell renal carcinoma (Hsp90 client: HIF-1 $\alpha$ /HIF-2 $\alpha$ )

HIF-1 and the highly homologous HIF-2 proteins function as nuclear transcription factors involved in the transactivation of numerous target genes, many of which are implicated in the promotion of angiogenesis and adaptation to hypoxia (for a review, see Harris, 2002). Although these proteins are normally labile and expressed at low levels in normoxic cells, their stability and activation increase several-fold in hypoxia. The molecular basis for the instability of these proteins in normoxia depends upon VHL, the substrate recognition component of an E3 ubiquitin lig-

**Table 1.** Possible/proposed uses of Hsp90 inhibitors in cancer therapy

Hsp90 inhibitors as single agents in situations in which Hsp90 client protein is necessary for cancer development or progression

- Bcr-Abl-positive leukemias
- Other leukemias/lymphomas dependent on a chimeric or mutated protein (e.g., Flt-3-dependent leukemias, anaplastic large cell lymphoma dependent on NPM/ALK)
- Androgen or estrogen receptor-dependent cancers (prostate and breast)
- Hormone-independent breast and prostate cancers in which receptor mutation can be documented
- Clear cell renal carcinoma in which pVHL is either inactive or deleted

Hsp90 inhibitors used to enhance response to chemotherapy

- taxol or doxorubicin in HER-2 or AKT over-expressing tumors (breast, ovarian, prostate and lung cancer)
- Gleevec in Bcr-Abl positive leukemias
- proteasome inhibitor in multiple myeloma



ase complex that targets HIF-1 and HIF-2 for proteasome-dependent degradation (Maxwell et al., 1999). Hypoxia normally impairs VHL function, thus allowing HIF to accumulate.

VHL can also be directly inactivated by mutation or hypermethylation, resulting in constitutive overexpression of HIF in normoxic cells. There are two clinical manifestations of VHL inactivation. In the first, hereditary von Hippel-Lindau disease arises from genetic loss of VHL, and affected individuals are predisposed to an increased risk of developing highly vascular tumors in a number of organs. This is due, in large part, to deregulated HIF expression and corresponding VEGF upregulation. A common manifestation of VHL disease is the development of clear cell renal cell carcinoma (CC-RCC) (Seizinger et al., 1988).

The second manifestation of VHL inactivation occurs in non-hereditary, sporadic CC-RCC. Interestingly, although mutations of VHL are found in most CC-RCCs (Gnarra et al., 1994), they are absent in other renal tumors, suggesting a dependence upon HIF activation specifically in RCC development. This is supported by the finding that all RCC-associated VHL mutants demonstrate defective ubiquitination of HIF (Ohh et al., 2000). Furthermore, in the majority of sporadic CC-RCC, HIF-1 overexpression was detected in tumors, but not in the corresponding normal kidney (Wiesener et al., 2001), and recent studies suggest that HIF-2 may also be a critical effector of CC-RCC (Kondo et al., 2002). Overall, the clinical data implicate HIF proteins as critical downstream targets of pVHL that play a major role in CC-RCC oncogenesis.

HIF interacts with Hsp90 (Gradin et al., 1996) and both GA and radicicol reduce HIF-dependent transcriptional activity (Hur et al., 2002; Isaacs et al., 2002; Minet et al., 1999). Hur et al. demonstrated that HIF from radicicol treated cells was unable to bind DNA, suggesting that Hsp90 is necessary for mediating the proper conformation of HIF and/or recruiting additional cofactors. Additionally, GA downregulates HIF expression by stimulating the protein's VHL-independent proteasomal degradation (Isaacs et al., 2002).

Functional inactivation of VHL occurs in approximately 70% of CC-RCC. While surgery is curative for localized disease, approximately one-third of patients present with metastatic disease, for which current treatments are largely ineffective. In order to examine the effectiveness of Hsp90 inhibition as a treatment modality in this disease, the National Cancer Institute is planning a phase II study of 17-AAG in RCC.

### Future challenges

From this brief overview, it should be apparent that Hsp90 inhibitors are unique in that, although they are directed toward a specific molecular target, they simultaneously inhibit multiple signaling pathways on which cancer cells depend for growth and survival, thereby perhaps circumventing the characteristic genetic plasticity that has allowed cancer cells to eventually evade the toxic effects of most molecularly targeted agents. However, the complex activity of such drugs will present many challenges, including how to clinically define the best use of Hsp90 inhibitors not only as single agents, but also when combined with other chemotherapeutics or signaling modulators (e.g., tyrosine kinase inhibitors, proteasome inhibitors, taxol, gemcitabine, and doxorubicin, to name several combinations that have shown preclinical efficacy). A more complete understanding of the biologic activity of Hsp90 and its many cochaperones should make this task somewhat easier.

### References

- Baselga, J., Norton, L., Albanell, J., Kim, Y.M., and Mendelsohn, J. (1998). Recombinant humanized anti-HER2 antibody (Herceptin) enhances the anti-tumor activity of paclitaxel and doxorubicin against HER2/neu overexpressing human breast cancer xenografts. *Cancer Res.* 58, 2825–2831.
- Basso, A.D., Solit, D.B., Munster, P.N., and Rosen, N. (2002). Ansamycin antibiotics inhibit Akt activation and cyclin D expression in breast cancer cells that overexpress HER2. *Oncogene* 21, 1159–1166.
- Blagosklonny, M.V., Fojo, T., Bhalla, K.N., Kim, J.S., Trepel, J.B., Figg, W.D., Rivera, Y., and Neckers, L.M. (2001). The Hsp90 inhibitor geldanamycin selectively sensitizes Bcr-Abl-expressing leukemia cells to cytotoxic chemotherapy. *Leukemia* 15, 1537–1543.
- Connell, P., Ballinger, C.A., Jiang, J., Wu, Y., Thompson, L.J., Hohfeld, J., and Patterson, C. (2001). The co-chaperone CHIP regulates protein triage decisions mediated by heat-shock proteins. *Nat. Cell Biol.* 3, 93–96.
- Csermely, P., Kajtar, J., Hollosi, M., Jalsovszky, G., Holly, S., Kahn, C.R., Gergely, P., Jr., Soti, C., Mihaly, K., and Somogyi, J. (1993). ATP induces a conformational change of the 90-kDa heat shock protein (hsp90). *J. Biol. Chem.* 268, 1901–1907.
- Gnarra, J.R., Tory, K., Weng, Y., Schmidt, L., Wei, M.H., Li, H., Latif, F., Liu, S., Chen, F., Duh, F.M., et al. (1994). Mutations of the VHL tumour suppressor gene in renal carcinoma. *Nat. Genet.* 7, 85–90.
- Gorre, M.E., Ellwood-Yen, K., Chiosis, G., Rosen, N., and Sawyers, C.L. (2002). BCR-ABL point mutants isolated from patients with STI571-resistant chronic myeloid leukemia remain sensitive to inhibitors of the BCR-ABL chaperone heat shock protein 90. *Blood* 100, 3041–3044.
- Gradin, K., McGuire, J., Wenger, R.H., Kvietkova, I., Whitelaw, M.L., Toftgard, R., Tora, L., Gassmann, M., and Poellinger, L. (1996). Functional interference between hypoxia and dioxin signal transduction pathways: competition for recruitment of the Arnt transcription factor. *Mol. Cell. Biol.* 16, 5221–5231.
- Grenert, J.P., Sullivan, W.P., Fadden, P., Haystead, T., Clark, J., Mimnaugh, E., Krutzsch, H., Ochel, H.J., Schulte, T.W., Sausville, E., et al. (1997). The amino-terminal domain of heat shock protein 90 (hsp90) that binds geldanamycin is an ATP/ADP switch domain that regulates hsp90 conformation. *J. Biol. Chem.* 272, 23843–23850.
- Grenert, J.P., Johnson, B.D., and Toft, D.O. (1999). The importance of ATP binding and hydrolysis by hsp90 in formation and function of protein hetero-complexes. *J. Biol. Chem.* 274, 17525–17533.
- Harris, A.L. (2002). Hypoxia—a key regulatory factor in tumor growth. *Nat. Rev. Cancer* 2, 38–47.
- Hernandez, M.P., Sullivan, W.P., and Toft, D.O. (2002). The assembly and intermolecular properties of the hsp70-Hsp-hsp90 molecular chaperone complex. *J. Biol. Chem.* 277, 38294–38304.
- Hur, E., Kim, H.H., Choi, S.M., Kim, J.H., Yim, S., Kwon, H.J., Choi, Y., Kim, D.K., Lee, M.O., and Park, H. (2002). Reduction of hypoxia-induced transcription through the repression of hypoxia-inducible factor-1 $\alpha$ /aryl hydrocarbon receptor nuclear translocator DNA binding by the 90-kDa heat-shock protein inhibitor radicicol. *Mol. Pharmacol.* 62, 975–982.
- Isaacs, J.S., Jung, Y.J., Mimnaugh, E.G., Martinez, A., Cuttitta, F., and Neckers, L.M. (2002). Hsp90 regulates a von Hippel Lindau-independent hypoxia-inducible factor-1  $\alpha$ -degradative pathway. *J. Biol. Chem.* 277, 29936–29944.
- Kelland, L.R., Sharp, S.Y., Rogers, P.M., Myers, T.G., and Workman, P. (1999). DT-diaphorase expression and tumor cell sensitivity to 17-allylamino, 17-demethoxygeldanamycin, an inhibitor of heat shock protein 90. *J. Natl. Cancer Inst.* 91, 1940–1949.
- Kondo, K., Kico, J., Nakamura, E., Lechpammer, M., and Kaelin, W.G., Jr. (2002). Inhibition of HIF is necessary for tumor suppression by the von Hippel-Lindau protein. *Cancer Cell* 1, 237–246.
- Maloney, A., and Workman, P. (2002). HSP90 as a new therapeutic target for cancer therapy: the story unfolds. *Expert Opin. Biol. Ther.* 2, 3–24.
- Maxwell, P.H., Wiesener, M.S., Chang, G.-W., Clifford, S.C., Vaux, E.C., Cockman, M.E., Wykoff, C.C., Pugh, C.W., Maher, E.R., and Ratcliffe, P.J.

- (1999). The tumor suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature* 399, 271–275.
- McLaughlin, S.H., Smith, H.W., and Jackson, S.E. (2002). Stimulation of the weak ATPase activity of human hsp90 by a client protein. *J. Mol. Biol.* 315, 787–798.
- Minet, E., Mottet, D., Michel, G., Roland, I., Raes, M., Remacle, J., and Michiels, C. (1999). Hypoxia-induced activation of HIF-1: role of HIF-1 $\alpha$ -Hsp90 interaction. *FEBS Lett.* 460, 251–256.
- Neckers, L. (2002). Hsp90 inhibitors as novel cancer chemotherapeutic agents. *Trends Mol. Med.* 8, S55–S61.
- Nimmanapalli, R., O'Bryan, E., and Bhalla, K. (2001). Geldanamycin and its analogue 17-allylamino-17-demethoxygeldanamycin lowers Bcr-Abl levels and induces apoptosis and differentiation of Bcr-Abl-positive human leukemic blasts. *Cancer Res.* 61, 1799–1804.
- Ohh, M., Park, C.W., Ivan, M., Hoffman, M.A., Kin, T.-Y., Huang, L.E., Pavletich, N., Chau, V., and Kaelin, W.G. (2000). Ubiquitination of hypoxia-inducible factor requires direct binding to the B-domain of the von Hippel-Lindau protein. *Nat. Cell Biol.* 2, 423–427.
- Oppermann, H., Levinson, A.D., Levintow, L., Varmus, H.E., Bishop, J.M., and Kawai, S. (1981). Two cellular proteins that immunoprecipitate with the transforming protein of Rous sarcoma virus. *Virology* 113, 736–751.
- Panaretou, B., Prodromou, C., Roe, S.M., O'Brien, R., Ladbury, J.E., Piper, P.W., and Pearl, L.H. (1998). ATP binding and hydrolysis are essential to the function of the Hsp90 molecular chaperone in vivo. *EMBO J.* 17, 4829–4836.
- Panaretou, B., Siligardi, G., Meyer, P., Maloney, A., Sullivan, J.K., Singh, S., Millson, S.H., Clarke, P.A., Naaby-Hansen, S., Stein, R., et al. (2002). Activation of the ATPase activity of Hsp90 by the stress-regulated cochaperone Aha1. *Mol. Cell* 10, 1307–1318.
- Pegram, M.D., Lipton, A., Hayes, D.F., Weber, B.L., Baselga, J.M., Tripathy, D., Baly, D., Baughman, S.A., Twaddell, T., Glaspy, J.A., and Slamon, D.J. (1998). Phase II study of receptor-enhanced chemosensitivity using recombinant humanized anti-p185HER2/neu monoclonal antibody plus cisplatin in patients with HER2/neu-overexpressing metastatic breast cancer refractory to chemotherapy treatment. *J. Clin. Oncol.* 16, 2659–2671.
- Prodromou, C., Roe, S.M., O'Brien, R., Ladbury, J.E., Piper, P.W., and Pearl, L.H. (1997). Identification and structural characterization of the ATP/ADP-binding site in the Hsp90 molecular chaperone. *Cell* 90, 65–75.
- Scheibel, T., and Buchner, J. (1998). The Hsp90 complex—a super-chaperone machine as a novel drug target. *Biochem. Pharmacol.* 56, 675–682.
- Schneider, C., Sepp-Lorenzino, L., Nimmesgern, E., Ouerfelli, O., Danishefsky, S., Rosen, N., and Hartl, F.U. (1996). Pharmacologic shifting of a balance between protein refolding and degradation mediated by Hsp90. *Proc. Natl. Acad. Sci. USA* 93, 14536–14541.
- Seizinger, B.R., Rouleau, G.A., Ozelius, L.J., Lane, A.H., Farmer, G.E., Lamiell, J.M., Haines, J., Yuen, J.W., Collins, D., Majoor-Krakauer, D., et al. (1988). Von Hippel-Lindau disease maps to the region of chromosome 3 associated with renal cell carcinoma. *Nature* 332, 268–269.
- Sharma, S.V., Agatsuma, T., and Nakano, H. (1998). Targeting of the protein chaperone, HSP90, by the transformation suppressing agent, radicicol. *Oncogene* 16, 2639–2645.
- Shiotsu, Y., Neckers, L.M., Wortman, I., An, W.G., Schulte, T.W., Soga, S., Murakata, C., Tamaoki, T., and Akinaga, S. (2000). Novel oxime derivatives of radicicol induce erythroid differentiation associated with preferential G(1) phase accumulation against chronic myelogenous leukemia cells through destabilization of Bcr-Abl with Hsp90 complex. *Blood* 96, 2284–2291.
- Siligardi, G., Panaretou, B., Meyer, P., Singh, S., Woolfson, D.N., Piper, P.W., Pearl, L.H., and Prodromou, C. (2002). Regulation of Hsp90 ATPase activity by the co-chaperone Cdc37p/p50cdc37. *J. Biol. Chem.* 277, 20151–20159.
- Solit, D., Zheng, F., Drobnjak, M., Munster, P., Higgins, B., Verbel, D., Heller, G., Tong, W., Cordon-Cardo, C., Agus, D., et al. (2002). 17-allylamino-17-demethoxygeldanamycin induces the degradation of androgen receptor and HER-2/neu and inhibits the growth of prostate cancer xenografts. *Clin. Cancer Res.* 8, 986–993.
- Stebbins, C.E., Russo, A.A., Schneider, C., Rosen, N., Hartl, F.U., and Pavletich, N.P. (1997). Crystal structure of an Hsp90-geldanamycin complex: targeting of a protein chaperone by an antitumor agent. *Cell* 89, 239–250.
- Whitesell, L., Mimnaugh, E.G., De Costa, B., Myers, C.E., and Neckers, L.M. (1994). Inhibition of heat shock protein HSP90-pp60v-src heteroprotein complex formation by benzoquinone ansamycins: essential role for stress proteins in oncogenic transformation. *Proc. Natl. Acad. Sci. USA* 91, 8324–8328.
- Wiesener, M.S., Munchenhagen, P.M., Berger, I., Morgan, N.V., Roigas, J., Schwartz, A., Jurgensen, J.S., Gruber, G., Maxwell, P.H., Loning, S.A., et al. (2001). Constitutive activation of hypoxia-inducible genes related to overexpression of hypoxia-inducible factor-1 $\alpha$  in clear cell renal carcinomas. *Cancer Res.* 61, 5215–5222.
- Xu, W., Mimnaugh, E., Rosser, M.F., Nicchitta, C., Marcu, M., Yarden, Y., and Neckers, L. (2001). Sensitivity of mature ErbB2 to geldanamycin is conferred by its kinase domain and is mediated by the chaperone protein Hsp90. *J. Biol. Chem.* 276, 3702–3708.
- Xu, W., Marcu, M., Yuan, X., Mimnaugh, E., Patterson, C., and Neckers, L. (2002). The chaperone-dependent E3 ubiquitin ligase CHIP mediates a novel degradative pathway for c-ErbB2/Neu. *Proc. Natl. Acad. Sci. USA* 99, 12847–12852.
- Young, J.C., and Hartl, F.U. (2000). Polypeptide release by Hsp90 involves ATP hydrolysis and is enhanced by the co-chaperone p23. *EMBO J.* 19, 5930–5940.